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Evaluation of Anti-Cancer Activity and Anti-Oxidant Status of *Calocybe Indica* (Milky Mushroom) On Dalton's Lymphoma Ascites Induced Mice

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ABSTRACT

Background: The background of the present study is to evaluate the anti-cancer and anti-oxidant activity of *Calocybe indica* (C.indica) against Dalton's lymphoma ascites (DLA) induced mice. Intraperitoneal administration of C. indica (250 mg/kg body weight) to DLA bearing mice showed significant changes in the body weight, hematological parameters, peritoneal ascites fluid, anti-oxidant status and lipid peroxidation in serum and liver tissue samples of the Swiss albino mice. Histopathological examinations of the liver tissue from the Swiss albino mice were analyzed. GC-MS (Gas Chromatography Mass Spectrography) analysis of C. Indica was tested for volatile compounds to support the C. indica possess anti-cancer and anti-oxidant activities. **Objective:** To evaluate the anti-cancer and anti-oxidant activity of *Calocybe indica* (C.indica) against Dalton's lymphoma ascites (DLA) induced Swiss albino mice. **Results:** The result reveals that, there is a significant increase in hematological parameters such as RBC count, hemoglobin and significant decrease in WBC counts in C. indica treated mice. The anti-oxidant status was measured on serum and liver tissue samples of the Swiss albino mice. The C. indica administration exhibits anticancer activity by modulating lipid peroxidation and reversed the histopathological changes in cancer bearing mice. GC-MS analysis of C. indica showed the evidences of phytochemicals such as o-Toluidine, 2,4-Xylidine, 3-Dodecene (Z), 2,6-Xylidine, Benzaldehyde 1-formylbenzo[b] Fluoranthene, anisaldehyde and benzaldehyde might possess the anticancer activity of C.indica. **Conclusion:**The present study suggests that C. indica possess anti-cancer and anti-oxidant activities and could be developed as an edible and potential (natural) anti-cancer agent

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INTRODUCTION

Mushrooms often called as queen of vegetables and table delicious and it technically confined to members of a family of fungi with gills. Edible mushrooms once called the "food of the gods". For millennia, mushrooms have been valued as edible and medical provisions for humankind, it play vital role in Indian economy and their popularity is increasing due to their delicacy and food value. Mushrooms is something special in the living world, being neither plants nor animals, they have been placed in a kingdom of their own called the kingdom of Myceteae. "Mushroom is a macro fungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand (Miles and Chang, 1997). Mushroom production is completely different from growing green plants, it do not contain chlorophyll and therefore depend on other plant material (the "substrate") for their food The greatest difficulty in feeding man is to supply a sufficient quantity of the body building material protein, but mushrooms provides valuable nutrients to the diet. They are excellent source of high quantity proteins compared to most vegetables. A good source of fibre, vitamin, low in fat with no cholesterol (Racz *et al.*, 1996). Mushrooms are not only sources of nutrients but have also been reported to have therapeutic values shown to be useful in preventing hypertension, hypercholesterolemia and also affecting the growth of tumor. Mushrooms are also known to have effective substances for antifungal, antiinflammatory, antitumor, antiviral, antibacterial, hepatoprotective, antidiabetic, hypolipemic, antithrombotic and hypotensive activities (Jong and Birmingham, 1992). Many naturally occurring substances have been tested for anti-tumor activity in experimental animals; such analyses have resulted in the availability of several effective anti-tumor drugs reviewed. This is because there is a clear

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relationship between oxidative stress, reductions in immune competency, resistance and survival against cancers and because mushrooms are known for eliciting anti-oxidant and anti-inflammatory effects in hosts (Ferreira *et al.*, 2010). They caught the attention of a significant number of investigators in the hopes of developing potent anti-tumor treatment regimens that could also have minimal adverse side effects that are often apparent with many traditional anti-oncolytic drugs. A large amount of compounds, including lectins, polysaccharides and polysaccharide-peptide as well as protein complexes, have been isolated from mushrooms and many of these have both immunomodulatory and anti-tumor effects (Mazumdar *et al.*, 1997). Among the large resources of fungi, higher Basidiomycetes especially mushrooms are unlimited sources of therapeutically useful biologically active agents. Great threat to human life by neoplastic diseases continues to increase and thus the pursuit for anti-tumor drugs takes a compelling urgency. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of a variety of human ailments (Zaidman *et al.*, 2005). The most significant medicinal effect of mushrooms and their metabolites that have attracted the attention of the public is their antitumor property (Wasser, 2007). The significant pharmacological effects and physiological properties of mushrooms are bioregulation (immune enhancement), maintenance of homeostasis and regulation of biorhythm, cure of various diseases and prevention and improvement from life threatening diseases such as cancer, cerebral stroke and heart diseases (Cohen *et al.*, 2002). Based on these earlier data, the present study was performed to evaluate the effect of using an ethanolic extract of *Calocybe indica* (*C.indica*) against Dalton's lymphoma (*in-vitro*) cancer cells that was introduced into a Swiss mouse model. Apart from effects on cancer-related parameters (body weight, ascites volumes) numerous measures of endpoints associated with anti-oxidant status in the hosts would also be evaluated in the cancer-injected hosts.

MATERIALS AND METHODS

Preparation of ethanolic extract of *C. indica*:

Freshly harvested *C. indica* fruiting bodies were procured from a mushroom farm in Coimbatore, India, and authenticated by a botanical expert Dr. A. Annamalai, Karunya University. The samples were washed thoroughly in sterile distilled water to remove adhering mud and extra-neous matter and damaged portions were removed. The samples were then dried at 55°C powdered and extracted in a Soxhlet apparatus using 70% ethanol. The residue obtained was dried in a vacuum desiccator, and the average yields for these experiments were found to be 12% [w/w]. Fixed amounts of the material were then removed and resuspended in 1 % (w/v) gum acacia at 500 mg extract/ml (stock solution) for subsequent dilution in the experiments below (Raja, 2012).

GC-MS analysis of bioactive components in ethanolic extract of *C. indica*:

Gas chromatography mass spectrometry (GC-MS) was used to identify phytoconstituents in the *C. indica* extract samples. Dried extract of *C. indica* was dissolved in 95% [v/v] ethanol and a 2 µl aliquot was injected into (and analyzed within) a GC Clarus 500 system (Perkin Elmer, Boston, MA) equipped with a Turbo mass gold-Perkin Elmer Detector and split injection system. Sample injector temperature was maintained at 250°C in the experiment. Mass spectral analyses were done at a 70 eV energy level and between 45 and 450 m/z, for the 45 min duration. Interpretation of the generated GC-MS spectra was done using a National Institute of Standard and Technology (NIST) database bearing >62,000 patterns. Specifically, the spectra of each unknown extract constituent was compared with that of known agents in the database and from this, the name, expected retention time (RT), molecular weight and molecular formula of the test constituent was ascertained (Gherman *et al.*, 2000).

Experimental Animals:

Male Swiss albino mice (8-10 week old, 25-30g) were purchased from the Agricultural University at Mannuthy (Trissur, India). All mice were kept in a pathogen-free air-controlled room maintained at 24°C with a 50% relative humidity and 12-hour light and dark cycle. All mice had *ad libitum* access to a standard diet (Hindustan Lever Ltd., Mumbai) and filtered water. All animal experiments were approved and performed according to the regulations of, the Institutional Animal Ethics Committee, Government of India [IAEC Reg No: IAECK/KU/BT/13/03].

After 1 week period to acclimatize, the mice were randomly divided into three groups of six mice each. Group I served as untreated normal control mice, Groups II and III were injected intraperitoneally (i.p.) with Dalton's lymphoma cells (1×10^6 cells/mice).

Group I = Normal mice
Group II = DLA Control
Group III = DLA + *C. indica*

Mice in Group III were treated alternatively with 250 mg/kg body weight *C. indica* extract/kg body weight (b.wt.) via i.p. injection. This dose was selected based on the acute toxicity study of the *C. indica* extract. Day of DLA induction was considered as day 1 and the experiment was terminated at day 15.

Dalton's lymphoma cells:

Dalton's ascitic lymphoma (DLA, a malignant highly invasive T-cell lymphoma of spontaneous origin) cells were received as a kind gift from the Tumor Research Centre (Adyar, Chennai, India). The DLA cells were maintained by serial i.p. transplantation of $\approx 1 \times 10^6$ viable cancer cells/mouse (in volumes of 0.25 ml of cells in PBS). Pilot studies indicated that mice injected with this level of DLA cells survived for 19–21 days (Goldie and Felix, 1951).

Acute toxicity studies:

An acute toxicity study was carried out according to OECD (Organisation for Economic Co-operation and Development) guidelines. Doses of ethanolic extract of *C. indica* ranging from 50–500 mg/kg body weight were administered i.p. to mice (six mice/dose group). All mice were then monitored continuously for 2 hours for any overt symptoms of toxicity (changes in behavior, tremors, sleep, coma) or death and then thrice daily for a period of 2 weeks (Halim *et al.*, 2011).

Determination of the effect of *C. indica* on body weight, ascites volume and hematologic parameters during Dalton's lymphoma ascites:

Throughout the experimental period body weight, food and water intake and general behavior of mice were monitored. One day after the final treatment (i.e., on treatment day 15), all mice were euthanized by cervical dislocation. At necropsy, body weight was recorded and then abdominal fluids were collected for measuring fluid volume. Blood collected at necropsy the hematologic parameters was assessed for total WBC, differential count, RBC counts and haemoglobin using standard counting techniques (D Amour *et al.*, 1965; Nemzek *et al.*, 2011).

Determination of the effect of *C. indica* on enzymatic and non-enzymatic anti-oxidant activity in mice induced with Dalton's lymphoma:

Serum samples (prepared from blood samples collected above) and liver tissue recovered at necropsy were assessed for activities of superoxide dismutase (SOD) (Kakkar *et al.*, 1984), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al.*, 1984) and glutathione-S-transferase (GST) (Habig *et al.*, 1974) as well as for levels of total glutathione (GSH) (Ellman, 1959), vitamins C (Omaye *et al.*, 1979) vitamins E, (Baker *et al.*, 1951) and protein (Lowry *et al.*, 1951). All enzymatic antioxidants were measured using the protein content present in the liver sample, which would allow each parameter to ultimately be expressed in terms of endpoint/mg protein.

Effect of *C. indica* on lipid peroxidation parameters in serum and liver samples of DLA - injected mice:

Lipid peroxidation in serum and liver samples of DLA injected mice were measured (Niehius and Samuelsson, 1968).

Histopathological analysis:

Liver tissues collected at necropsy were fixed in 10% formalin solution. After fixation, the tissues were embedded in paraffin and sections cut at 5 μ m to later be stained with hematoxylin and eosin. The sections were then examined under light microscope and photographed (Standish *et al.*, 2006).

Statistical analysis:

Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). All data are expressed as mean (\pm SD); p-values < 0.05 were considered statistically significant.

Results:**GC-MS analysis of bioactive components in ethanolic extract of *C. indica*:**

The ethanolic extract of *C. indica* was found to contain the chemical phytoconstituents which is shown in (Table 1). *C. indica* could be attributed due to the higher content of aromatic amines and many phenolic compounds such as *o*-Toluidine, 2,4-Xylidine, 3-Dodecene (Z), 2,6-Xylidine, Benzaldehyde 1-formylbenzo[b] Fluoranthene, anisaldehyde and benzaldehyde. These compounds are thought to contribute the antitumour characteristic of the mushrooms

Acute toxicity studies of *C. indica*:

All treated mice treated with different dose of *C. indica* showed no overt behavioral changes or mortality after a single injection of up to 250 mg extract/kg body weight over the 2 weeks observation period. The summary of the results were shown in Table 2. Some clear changes were noted in the physical appearance and behavior of mice in the groups that received doses ≥ 300 mg extract/kg body weight, but no lethality was ever

seen in this 2 weeks period. Hence, the LD₅₀ values of *C. indica* for Swiss albino mice were found to be 250 mg extract/kg body.

Table 1: Bioactive components identified in ethanolic extract of *C. indica* by GC-MS analysis.

S.No.	Compound	Retention Time (RT)	Formula	Molecular weight
1	<i>o</i> -Toluidine	6.23	C ₇ H ₉ N	107
2	2,4-Xylidine	7.17	C ₈ H ₁₁ N	121
3	3-Dodecene (Z)	7.18	C ₁₂ H ₂₄	168
4	2,6-Xylidine	7.19	(CH ₃) ₂ C ₆ H ₃ NH ₂	121
5	1-Tetradecene	10.60	C ₁₄ H ₂₈	196
6	Cyclohexadecane	13.78	C ₁₆ H ₃₂	224
7	2-Methyl propanol	15.19	C ₄ H ₁₀ O	74
9	Benzaldehyde	16.18	C ₇ H ₆ O	106
10	1-formylbenzo[b]fluoranthene	20.99	C ₂₁ H ₁₂ O	280
11	Anisaldehyde	28.04	C ₈ H ₈ O ₂	136
12	7-Hydroxy-1-methoxy-6-methylanthraquinone	29.18	C ₁₆ H ₁₂ O ₄	268

Table 2: Acute toxicity studies.

Parameters Monitored	Dose of <i>C.indica</i> provided in single injection (mg/kg body weight)									
	50	100	150	200	250	300	350	400	450	500
Changes in skin	-	-	-	-	-	-	+	+	+	+
Changes in fur	-	-	-	-	-	-	+	+	+	+
Changes in eyes	-	-	-	-	-	+	+	+	+	+
Changes in mucous membranes	-	-	-	-	-	-	-	-	+	+
Behavior Patterns	-	-	-	-	-	+	+	+	+	+
Tremors	-	-	-	-	-	-	-	-	+	+
Sleep	-	-	-	-	-	-	-	+	+	+
Coma	-	-	-	-	-	-	-	-	-	+
Death	-	-	-	-	-	-	-	-	-	-

Mice were monitored for 2 weeks post-injection: (+) abnormal, (-) normal

Changes in the body weight of Dalton's lymphoma ascites bearing mice after *C. indica* treatment:

The effect of *C. indica* extracts on body weight of the mice was shown in the figure 1. The body weight of DLA alone induced mice was found to be significantly ($p < 0.05$) increased (37.65 ± 0.31^b) when compared to normal mice (29.69 ± 0.84^a). Treatment of *C. indica* extracts in DLA induced mice significantly ($p < 0.05$) decreased the bodyweight to 35.15 ± 0.21^d respectively, when compared to DLA control mice.

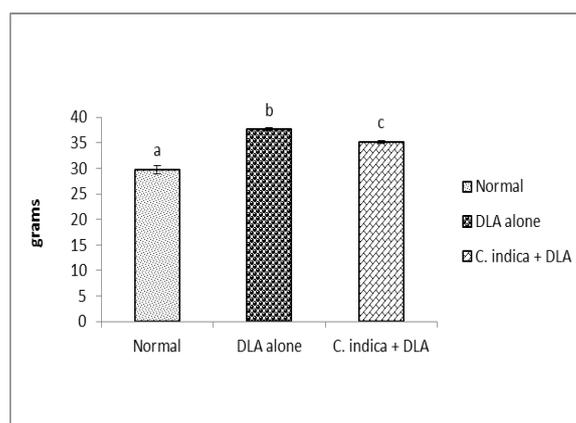


Fig. 1: Values are mean \pm SD for 6 mice/group. (a,b & c) Values not sharing a common superscript significantly differ at $p < 0.05$ (DMRT).

Changes in the tumor fluid volume of Dalton's lymphoma Ascites bearing mice after *C. indica* treatment:

The effects of *C. indica* extract on ascites tumor fluid of the mice were shown in the figure 2. The volume of ascitic tumor fluid for DLA alone induced mice was found to be significantly ($p < 0.05$) increased (12.95 ± 0.42^a) when compared to normal mice. Treatment of *C. indica* extracts in DLA induced mice significantly ($p < 0.05$) decreased the volume of ascitic tumor fluid to 07.92 ± 0.53^c respectively, when compared to DLA control mice.

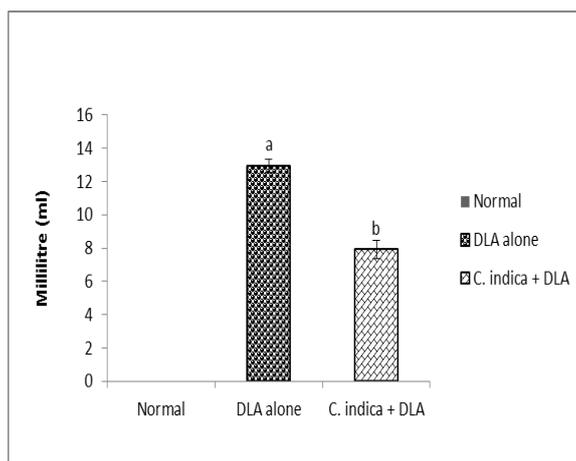


Fig. 2: Values are mean \pm SD for 6 mice/group. (a,b & c) Values not sharing a common superscript significantly differ at $p < 0.05$ (DMRT).

Changes in the total RBC of Dalton's lymphoma ascites bearing mice after *C. indica* treatment:

The effects of *C. indica* extract red blood cell count mice were shown in the figure 3. The red blood cell count for DLA alone induced mice was found to be significantly ($p < 0.05$) decreased (3.43 ± 0.29^b) when compared to normal mice (11.34 ± 0.27^a). Treatment of *C. indica* extracts in DLA induced mice significantly ($p < 0.05$) increased the red blood cell count of the mice to (8.31 ± 0.11^d) respectively, when compared to DLA control mice.

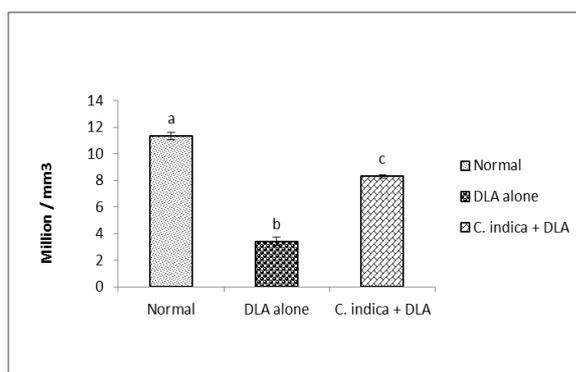


Fig. 3: Values are mean \pm S.D for the six mice in each group. Values are not sharing a common superscript letter (a, b & c) differ significantly at $p < 0.05$ (DMRT).

Changes in the total WBC count of Dalton's lymphoma ascites bearing mice after *C. indica* treatment:

The effects of *C. indica* extract white blood cells count mice were shown in the figure 4. The white blood cell count for DLA alone induced mice was found to be significantly ($p < 0.05$) increased (12.12 ± 0.21^b) when compared to normal mice (7.21 ± 0.21^a). Treatment of *C. indica* extracts in DLA induced mice significantly ($p < 0.05$) decreased the white blood cell count of the mice to (2.17 ± 0.31^c) respectively, when compared to DLA control mice.

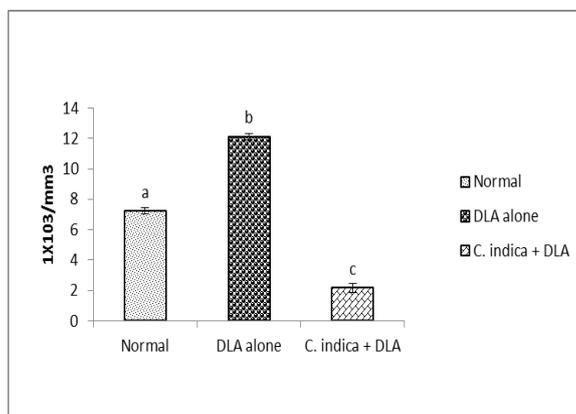


Fig. 4: Values are mean \pm S.D for the six mice in each group. Values are not sharing a common superscript letter (a, b & c) differ significantly at $p < 0.05$ (DMRT).

Changes in the total Hemoglobin level in the blood of Dalton's lymphoma ascites bearing mice after *P. ostreatus* and *C. indica* treatment:

The effects of *C. indica* extract in Hemoglobin level of mice were shown in the figure 5. The Hemoglobin level for DLA alone induced mice was found to be significantly ($p < 0.05$) decreased (8.29 ± 0.38^b) when compared to normal mice (14.9 ± 0.26^a). Treatment of *C. indica* extracts in DLA induced mice significantly ($p < 0.05$) increased the Hemoglobin level of the mice (13.79 ± 0.35^d) respectively, when compared to DLA control mice.

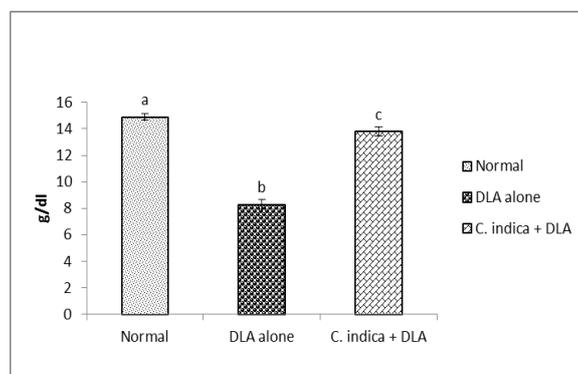


Fig. 5: Values are mean \pm S.D for the six mice in each group. Values are not sharing a common superscript letter (a, b & c) differ significantly at $p < 0.05$ (DMRT).

Effect of *C.indica* on enzymatic and non-enzymatic anti-oxidants in serum of Dalton's lymphoma ascites bearing mice:

Enzymatic and non-enzymatic anti-oxidant related parameters, such as SOD, CAT, GPx and GST activities, as well as levels of GSH, vitamin C and vitamin E in the sera of mice in each experimental group is presented in Table 3. As readily evident, by treatment Day 15, mice that had been injected only with DLA (Group II) displayed significant reductions in each of the measured parameters. On average, these reductions (relative to levels in normal mice) ranged from 53% (for GSH levels) to 82% (for GST activity), with the remainder ranging from 64-76%. These changes were significantly reversed in *C. indica*-treated mice with reductions (albeit still significant) vs. control values ranging from 15-33% now. It is interesting to note that of the parameters that had been most affected in mice that had the DLA treatment only, which is GSH and GST, these parameters were the most abated or reversed by the *C. indica* extract treatment. In the *C. indica* extract alone treated mice the activities of the enzymatic and non-enzymatic antioxidants were close to the normal values.

Table 3: Enzymatic and non-enzymatic anti-oxidant parameters in serum of the experimental mice.

TREATMENTS	SOD	CAT	GPx	GSH	GST	VIT - C	VIT -E
Normal	5.60 \pm 0.17 ^a	4.09 \pm 0.08 ^a	6.07 \pm 0.68 ^a	7.71 \pm 0.20 ^a	0.50 \pm 0.05 ^a	2.11 \pm 0.03 ^a	3.71 \pm 0.12 ^a
DLA control	1.98 \pm 0.16 ^b	0.98 \pm 0.15 ^b	2.20 \pm 0.29 ^b	3.59 \pm 0.35 ^b	0.09 \pm 0.01 ^c	0.75 \pm 0.01 ^b	0.92 \pm 0.13 ^b
DLA + <i>C. indica</i>	4.40 \pm 0.73 ^c	3.04 \pm 0.41 ^c	4.29 \pm 1.50 ^c	6.79 \pm 0.82 ^c	0.41 \pm 0.04 ^b	1.79 \pm 0.15 ^c	2.48 \pm 0.21 ^c

Units: SOD - 50% inhibition of NBT min/mg/protein; CAT - μ moles of H₂O₂ consumed min/mg/protein; GPx- μ g/min/mg protein; GSH - μ g/min/mg/protein; GST - μ moles of CDNB conjugated/min/mg protein; VIT C - U/mg protein; VIT E - μ M/mg serum.

Values are mean \pm SD for six mice/group. (a, b & c) Within a given parameter, values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Effect of *C.indica* on enzymatic and non-enzymatic anti-oxidants in liver tissue of Dalton's lymphoma ascites bearing mice:

Enzymatic and non-enzymatic anti-oxidant parameters in the livers tissues of the experimental mice are shown in Table 4. As in the sera, there were significant decreases in all of the enzymatic and non-enzymatic anti-oxidant related parameters as a result of DLA induction. Again, the treatments with the *C. indica* extract on each of the significantly reversed the effects on each of the measured parameters due to cancer.

Table 4: Enzymatic and non-enzymatic anti-oxidant parameters in liver samples of the experimental mice.

TREATMENTS	SOD	CAT	GPx	GSH	GST	VIT - C	VIT -E
Normal	18.06 \pm 0.51 ^a	30.88 \pm 0.85 ^a	39.61 \pm .05 ^a	21.98 \pm 0.14 ^a	8.96 \pm 1.01 ^a	45.40 \pm 0.62 ^a	35.55 \pm 0.22 ^a
DLA control	9.26 \pm 0.24 ^b	19.10 \pm 0.5 ^b	24.90 \pm .95 ^b	8.58 \pm 0.19 ^b	2.65 \pm 1.01 ^b	34.22 \pm 1.64 ^b	26.12 \pm 0.26 ^b
DLA + <i>C. indica</i>	16.94 \pm 0.46 ^c	27.19 \pm 0.69 ^c	32.50 \pm 0.30 ^c	18.85 \pm 0.07 ^c	5.54 \pm 1.31 ^c	41.80 \pm 1.57 ^c	30.81 \pm 1.63 ^c

Units: SOD - 50% inhibition of NBT min/mg/protein; CAT - μ moles of H₂O₂ consumed min/mg/protein; GPx- μ g/min/mg protein; GSH - μ g/min/mg/protein; GST - μ moles of CDNB conjugated/min/mg protein; VIT C - U/mg protein; VIT E - μ M/mg serum.

Values are mean \pm SD for six mice/group. (a,b & c) Within a given parameter, values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

Effect of *C.indica* on serum and liver tissue lipid peroxidation (LPO) status:

Effects of *C. indica* extract on serum and liver LPO are shown in Figure 6. In mice that received the DLA only, LPO levels were found to be 7.34 ± 0.8^b nmole/ml serums and 18.86 ± 1.6^b nmole/mg liver. These were significantly elevated relative to levels seen in normal control mice that are 1.11 ± 0.08^a nmole/ml serum and 10.91 ± 2.4^a nmole/mg liver. Again, treatment of DLA injected mice with *C. indica* appeared to have a mitigating effect as LPO levels were significantly reduced by 48% to 5.77 ± 0.02^c nmole/ml serum and 37% to 15.16 ± 0.53^c nmole/mg liver. Both in serum and liver tissue, the LPO levels of *C. indica* extract alone treated mice was close to that of normal values.

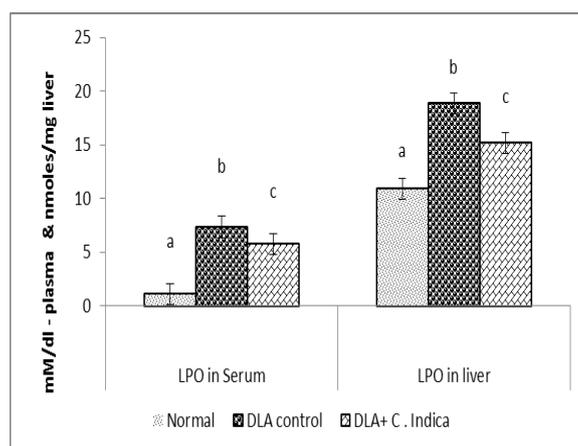
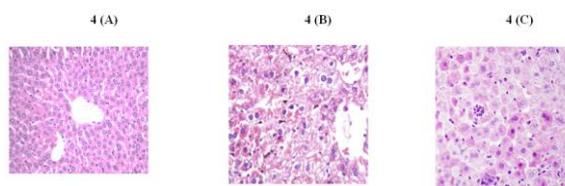


Fig. 6: Values are mean \pm S.D for the six mice in each group. Values are not sharing a common superscript letter a,b & c differ significantly at $p < 0.05$ (DMRT)

Effect of *C.indica* on liver histopathology of Dalton's lymphoma ascites bearing mice:

The effect of *C.indica* on liver histopathology of Dalton's lymphoma ascites bearing mice shown in Figure 4, (Figure 4 A) The liver tissue of normal mice showed normal hepatocytes each with well-preserved cytoplasm, a prominent nucleus and nucleolus, and the organ had a normal central vein. (Figure 4B) The liver of DLA only-treated mice, there was severe hepatic fibrosis and inflammatory cell infiltration. In contrast to this, in the liver of lymphoma-injected mice and mild degree of leukocyte infiltration, some bile duct proliferation, and indications of calcification (Figure 4C).



H&E-stained liver sections are shown (100X magnification):

(A) Liver of normal mice (normal architecture). (B) DLA only-treated hosts (loss of normal architecture with presence of cancer cells along and increased lymphoid aggregation). (C) Presence of scattered lymphoid series with fewer neoplastic cells in DLA-treated mice with *C. indica* (hepatocyte necrosis was highly reduced).

Discussion:

The therapeutic potential of mushrooms has become important in the field of chemotherapy. Previous reports have shown that mushrooms could impart anti-oxidant, anti-inflammatory and anti-tumor activities in *in-vivo* and *in-vitro* studies (Thekkuttuparambil *et al.*, 2007; Song *et al.*, 2013). The antitumor activity of mushroom relies on the phytochemicals such as aromatic amines, terpenoids and phenolic compounds in the ethanolic extract of *C.indica* are in accordance with the previous reports (Lindequist., 2005). The present study showed that administration of an ethanolic extract of *C. indica* to DLA injected mice led to significant reduction in

several parameters associated with cancer progression. A 14-day treatment with the *C. indica* ethanolic extract significantly decreased the presence of DLA cells in cancer-injected mice. This suggested that the *C. indica* apparently led to effective reductions in the multiplication of these cancer cells and ultimately, suppressed the malignant course of events. The anti-cancer effect of the *C. indica* could have been due, in part, to its rich anti-oxidant content and the presence of polyphenols (see Table 1). Many studies have shown that ingestion of foods or treatment with preparations from foods rich in anti-oxidants (including flavonoids and polyphenols) resulted in prevention of cancer development and spread in a treated host (Romagnolo and Selmin, 2012; Sriram *et al.*, 2010).

The protective effect of *C. indica* against the deleterious effect of the lymphoma was also reflected in reduction on body weight and ascitic fluid relative to values in mice that had only received the DLA. As would be expected, the increase in body weight in the latter hosts would be attributable primarily to accumulation of peritoneal fluid as the cavity enlarged due to an increased number of cancer cells and ascites fluid(Christinaa *et al.*, 2003).

Indeed, administration of the *C. indica* significantly reversed the hemoglobin content, RBC levels to near normal. The total leukocytes (WBC) level found to be increased due to the invasion of lymphoma cells in the cancer bearing mice. During cancer progression, large amount of blood cells are necessary to meet the ever-increasing high requirement of nutrients to support rapid development and proliferation.

Similarly, lymphopenic states were noted in Swiss mice that received an injection of an ascitic lymphosarcoma (Thakur *et al.*, 2005) While those authors attributed the increases in WBC levels to the host mounting an immune response to the cancer cells, that same study also indicated that the route of cancer cell injection (Intraperitoneally vs. subcutaneous) impacted on the temporal patterns of lymphopenia, i.e., using either route resulted in an initial spike (up to around 14 days post-injection) in splenic WBC contents, but after this point there were significant declines in WBC levels in this organ in hosts that had been injected Intraperitoneally.

Excessive generation of reactive oxygen species has been considered as hallmark in several cancers, including Dalton's ascitic tumor model (Kumar *et al.*, 2012) Anti-oxidant enzymes that scavenge inter mediates of oxygen reduction provide a primary defense against free radicals *in situ*. It is well known that SOD, CAT and GPx also play important roles as protective enzymes against LPO in tissues (Raj Kapoor *et al.*, 2003) Several investigators reported that reduced activities of SOD, CAT and GPx in tumor-bearing animals may be due to a down-regulation of SOD and CAT genes induced by certain hormonal factors or ROS themselves (Quan *et al.*, 2011). In keeping with those findings, the present study indicated a reduction in the activities of SOD, CAT, and GPx in serum and liver tissues of DLA-injected mice. Administration of *C. indica* increased the activity of anti-oxidant enzymes in order to counteract the accumulation of carcinogens generated in serum and liver of DLA injected mice. This suggested that either the extract itself contained sufficient levels of anti-oxidants to counter those being produced by the growing lymphoma cells and the presence of phytochemicals in the extract plays directly or indirectly led to increases in expression of the key anti-oxidant enzymes in the DAL injected mice.

Reduced glutathione, a substrate for GPx that also can act alone to neutralize hydroxyl radicals and singlet oxygen, is present in high concentration in cells for protection from free radical attack. In the present study, the decreased levels of GSH in DLA-injected mice (Group II) may have been due to excess utilization of this anti-oxidant by resident cells against lymphoma-generated ROS as well as by the cancer cells to assure their own survival and subsequent proliferation. Because reduced GSH also helps to maintain cellular levels of vitamins C and E in their active forms which then allows each to partake in neutralizing free radicals (Quan *et al.*, 2010) when there are reductions in levels of GSH, cellular levels of ascorbic acid and tocopherol are also lowered (Erat *et al.*, 2007).

The over-consumption of GSH in mice with the DLA cells may be a major reason underlying the concurrent observed decreases in vitamin C and E levels in the DLA-injected mice here. These states were reversed in *C. indica* treated mice. As above, this suggested that the extract may have contained sufficient levels of anti-oxidants to counter the ROS generated by the growing lymphoma cells contained agents that indirectly led to increases in expression of key anti-oxidant enzymes by cells in the injected hosts, the reductions in ROS levels would have meant less direct consumption and interaction of GSH with ROS and also less use of GSH by GPx in reparations in local cells induced by per oxidative damage in cell membranes caused by free ROS in the DLA-injected hosts.

The significant increases in levels of LPO observed in serum and liver of DLA-injected mice may have been due to the ROS radicals that were produced by the unfold population of DLA cells and by the ever increasing lack of the key free radical scavenging enzymes assessed (Singh *et al.*, 2006; Singh *et al.*, 2000). The preventive or mitigative effect of the *C. indica* against formation of LPO seen here suggested that the extract might have had a direct effect on membranes in a manner that decreased their susceptibility to ROS (Maiti *et al.*, 2011; Jang *et al.*, 2010). The lower presence of ROS (for many of the reasons outlined above) and the increased ability of cells to utilize GPx pathways to reverse peroxidative damage could also explain the

decreases in LPO in extract-treated hosts that have acquired the DLA. In light of these outcomes, it is not surprising that histological examination of livers of DLA-injected mice showed marked changes that were normalized in hosts given the *C. indica*.

Conclusion:

The ethanol extract of *C. indica* was shown here to be effective in inhibiting cancer growth (i.e., in an ascitic tumor model), as well as mitigating and reversing many pathologic states (anemia, lymphopenia, hepatic damage, etc.) associated with growth of this cancer in mice. Data here suggested that this anti-cancer activity resulted, in part from increases in enzymatic and non-enzymatic anti-oxidant levels. Also these activities of *C. indica* could be attributed due to the higher content of aromatic amines and many phenolic compounds such as aldehyde, ketones, terpenonids, toluidine, xylidine, cyclohexadecane, 7-hydroxy-1-methoxy-6-methylanthroquinone, 6-aminomethyl 2-naphthol and benzaldehyde. Based on the finding of the present study, it may be suggested that the edible mushroom *C. indica* could be used as a potential (natural) treatment for cancer therapy.

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The authors declare no conflicts of interest. The authors alone are responsible for the content of this paper.

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